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POLYMERIC COMPOSITIONS AND SENSOR DEVICES.

This invention relates to new polymeric compositions, and more particularly to polymeric compositions having improved anti-fouling properties which make them useful for surface coatings but especially for semi-permeable membranes and analytical sensor devices containing them.

A wide variety of products and items of apparatus made from or containing polymers have been devised for use in environments which may contain components which can have an adverse effect on their performance and reliability.

This effect becomes especially evident in the problems which arise for bio-compatibility - i.e. compatibility with fluids and samples of biological origin, e.g. blood - as materials such as proteins and the like tend to cling to the the surface of the polymer and contaminate it. This effect (known as "fouling") seriously - and often rapidly - reduces the ability of polymers to continue to function as desired. Furthermore, this "fouling" effect is usually irreversible so fouled polymer surfaces cannot be cleaned and used again.

Bio-compatibility can be required in many products, as diverse as contact lenses and working surfaces, but we have found it to be especially troublesome in the field of sensor devices for use in the analysis of biological media. For this reason, the description in this specification deals in greatest detail with sensor devices and their use, but most of the detail we describe is applicable also to the many other uses and products which call for bio-compatibility.

Thus, there is a need to find materials which have good and satisfactory compatibility with media containing high molecular weight compounds as well as colloidal and cellular suspensions."

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So far as sensor devices are concerned, it is known to make and use a variety of electrolytic sensor devices incorporating one or more electrodes to produce a signal output from which specific analytes can be detected and measured. These electrodes can act in several ways and can detect a variety of chemical species, for example by detecting such conditions as oxidation, reduction, acidity/alkalinity (pH), electrical potential and current flow. In known sensor devices it is conventional to use, between the electrode and the sample under examination, a permeable or semi-permeable membrane which acts as a barrier to restrict the diffusion of components from the sample towards the electrode. This can serve to avoid concentrations of analyte at the electrode reaching levels which are too high to allow the measurements to be sufficiently accurate, but especially it can be selective - so that it allows desired components (i.e. those to be detected or measured) to pass through to the electrode while holding back or reducing the passage of undesirable components -- e.g. compounds which can interfere with the accuracy of the measurements made at the electrode of the desired analyte components.

As it is unusual for materials to possess a combination of the various properties desired, to secure a desired overall effect it is often necessary to use combinations of two or more different membranes - each conferring some of the properties desired. Typically, for a practical system, two membranes are used - an outer and an inner - the outer one providing the required compatibility to interface with the sample to which it is exposed while the inner one provides the selectivity required.

Detection and measurement of glucose is particularly important, as this compound is a vitally important

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component of biological systems and media, for example blood, and also a common component of industrial media. Consequently, there is a great demand for reliable sensor devices for monitoring the sugar (glucose) content of a wide variety of samples.

Many membrane materials have been proposed and some are widely used (e.g. polycarbonate and surfactant-modified cellulose acetate) but, though these can be suitable for a majority of uses, they are subject to irreversible fouling when exposed to complex sample matrices. This results in a membrane or barrier, when fouled, reducing the amounts of solute able to pass through it satisfactorily and consequently that conventional membranes tend to have a short operating life when the sample under examination contains appreciable amounts of materials of high molecular weight, etc., and there is a strong need for a form of membrane or barrier which is more resistant to this fouling effect.

We have now found that these problems can be considerably reduced by new compositions containing mixed polymers, one of which is a poly-vinylpyridine, as these new compositions have the property of reversible fouling so that any fouling discussed above can be removed by washing and the polymer re-used.

Thus according to our invention we provide new polymer compositions characterised in that they are composed of an impermeable polymer together with a poly-vinylpyridine ("PVP").

The impermeable polymer and PVP are preferably mixed intimately, to achieve uniformity of behaviour throughout it and avoid undesirable irregularities.

According to a further feature of our invention we also provide an improved sensor device comprising means for detecting components present in fluid samples and providing

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an output representative of the content of said component and a permeable membrane interposed between the detecting means and a sample to be examined, characterised in that the membrane is made of a composition containing an impermeable polymer and a poly-vinylpyridine ("PVP").

The impermeable polymer component may be any of various polymers and polymer compositions, for example polymers and copolymers of halogenated monomers (e.g. one or more of the halogenated hydrocarbon monomers, especially chlorinated derivatives of ethylene), polyaryl or polyarylether sulphones, polyethylene, polypropylene, polyurethanes, polycarbonates, cellulose, cellulosic materials, and the like, or mixtures thereof.

The polyvinyl chloride (PVC) may be any polymer of vinyl chloride, as for example those made and available commercially. the molecular weight of the PVC is relatively non-critical to the present invention; most commercial grades can be used satisfactorily, and the grade most appropriate for any particular use can be determined readily by simple trial. A typical and convenient molecular weight is in the range 10,000 to 200,000 but products outside this range and mixtures of different molecular weight materials may be used if desired.

The polyaryl sulphone (PS) may be a sulphonated or an un-sulphonated material, or a mixture of two or more thereof. It may be used in a variety of forms, for example a product comprising mainly one structure fitting the description "polyaryl sulphone" or it may be a mixture of such components having different structure or molecular configuration characteristics. Conveniently, it may be a commercially available product.

The polyaryl sulphone may be, for example, any of those compounds disclosed and more fully described in European Patent No. 225094, referred to therein as

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"sulphonated or un-sulphonated polyaryl sulphones" and, in abbreviated form, as "PAS." These are described as polymers containing repeating units of the general formula $-(-\text{Ar}-\text{Y})-$ wherein Ar represents a divalent aromatic radical and optionally, but preferably, at least some of the Ar groups are sulphonated, and Y represents $-\text{SO}_2-$. The group ar is preferably a group containing at least two aromatic rings fused together or linked together by a direct bond or linked together by an aliphatic group, an oxygen atom or sulphur atom or a sulphone group. The details and disclosures of that European patent concerning the sulphone polymers is incorporated herein by reference, including the statement that some of the said polymers or copolymers are disclosed in and can be made by methods described in European Patent No. 8894.

The choice of polymers can vary according to the particular use or environment in which it is to be used, but the choice can be made readily by simple trial.

The preferred impermeable polymer is polyvinyl chloride ("PVC") but other polymers may be used in place of PVC. The impermeable polymer is conveniently referred to as a "support" polymer because the PVP serves as an additive and cannot achieve the desired result alone.

Thus according to a preferred feature of our invention we provide novel compositions containing polyvinyl chloride ("PVC") and poly-vinylpyridine ("PVP").

Also, according to a further feature of our invention, we provide an improved sensor device comprising means for detecting components present in fluid samples and providing an output representative of the content of said component and a permeable membrane interposed between the detecting means and a sample to be examined, characterised in that the membrane is made of a composition containing polyvinyl chloride ("PVC") and a poly-vinylpyridine ("PVP").

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The description herein is principally addressed to PVC because this is the preferred impermeable polymer, but the details given in this description should be read as applying to other impermeable polymers unless they are
5 clearly peculiar to PVC itself.

The novel compositions of our invention can be made in any desired form, but a particularly useful form is that of a membrane.

For uses other than sensors, the compositions of our
10 invention can be in any appropriate form - most commonly and conveniently as a coating, which may be attached to the surface of a polymer article or product by conventional means. This attachment can be done most conveniently by deposition from a solution of our composition in a solvent
15 and then removing the solvent by evaporation.

The detecting means is most conveniently of one of an electrochemical nature, but other types (e.g. optical or spectrophotometric detecting systems) may be used if desired. The preferred detecting means is an electrolytic
20 detection device. This usually comprises an active electrode operating in a liquid or gel phase electrolyte-containing medium. In most applications the electrolyte will be aqueous (i.e. aqueous or aqueous-based) and may be provided by the sample by diffusion, but the use of non-
25 aqueous electrolyte media (for example organic-based media) is not excluded.

The active electrode may be any of those known in the art, for example a metal electrode, but especially an electrode made of platinum. Usually the active electrode
30 will function as an anode. This is most conveniently made or used in combination with a silver/silver chloride reference electrode, as for example in the so-called Clark electrode, which commonly comprises a platinum electrode surrounded by a silver/silver chloride ring.

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The system may, if desired, contain an enzyme which can act to convert one analyte into another, for ease of detection.

According to our invention we also provide a method
5 for determining a component in a fluid sample, which comprises contacting the sample with a sensor device as defined above.

The invention is especially useful for electrolytic analysis procedures, as discussed above.

10 If desired, these membrane compositions may be used in conjunction with additional materials, especially membranes made of other materials, for example as multi-layer membrane products - provided that at least one layer is
15 formed of the composition of polyvinyl chloride and polyvinylpyridine. Such additional layers of other materials may be used to add further desirable properties to those of the PVC/PVP (polyvinyl chloride/poly-vinylpyridine) composition of the present invention. Such materials may be of appropriate selective permeability to regulate the
20 access of components before or after passage through the composition of polyvinyl chloride and poly-vinylpyridine, and/or have a physical form or strength which protects the composition of polyvinyl chloride and poly-vinylpyridine from damage or provides it with any desired degree of
25 stability of shape or positioning in use.

The sensor device and method of our invention may be applied to the analysis of samples for the detection or determination of a variety of analyte compounds, especially low molecular weight, non-volatile substrates, for example
30 sugars and neutral phenols.

This improved membrane material according to the present invention is especially applicable to sensor devices and methods of analysis for the monitoring, measurement and assessment of biological media in which

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glucose is present, for example blood and other biological (e.g. bodily) fluids, because it has good permeability towards glucose.

The working electrode may be any of those known in the art as useful for electrolytic analytical work, especially those functioning by electrolytic oxidation (i.e. as anodes). Such an electrode may be made of any material with an electrochemically active surface, for example any of those used in electrochemical analysis where the surface is electrochemically polarised. The electrode may be in any conventional form, for example sheet or wire, or as a coating deposited upon a substrate. The modes of using the sensor device may be any of those conventionally used with the electrode system employed.

Among the proposed sensor devices which have been proposed for carrying out such monitoring and measurement, many have contained enzymes - which act on the substrate chemical being evaluated and generates a different chemical which can be determined, thus providing means for determining the substrate chemical indirectly. Especially, glucose oxidase has been used because it catalyses oxidation of glucose to gluconic acid -- producing hydrogen peroxide via oxygen reduction. The hydrogen peroxide is very readily and conveniently determined electrolytically.

The preferred electrode material for use in direct electrolytic measurements of glucose is platinum, but other forms and materials known for use as sensors may be used if desired, and may be chosen according to the particular analyte and system concerned.

Polyvinyl chloride (PVC) is a well known material, and may be made by polymerising vinyl chloride. The forms used for making the compositions for the composite membrane in this invention may be any polymer of vinyl chloride, and are preferably those made and available commercially.

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These products may include co-polymers.

Preferably the PVC should be free from any added plasticiser (an additive ingredient which is often present in commercial products intended for uses such as moulding).

5 The presence of a commercially used plasticiser is not necessarily harmful and can be tolerated if it does not interfere unduly with the properties of the mixed PVC/PVP polymer compositions. However, a commercial plasticiser is not required and it is easier to control the properties of
10 the PVC/PVP composition if one does not have to make allowance for the possible effects of any commercially added ingredient - the nature of which may not be known or declared by the supplier of the PVC.

Such "un-plasticised" PVC polymers are readily
15 obtainable in commerce, however, and it is necessary only for the quality and purity of any polymer to be checked, whether by its specification or labelling. The molecular weight of the PVC is relatively non-critical, and most commercial grades will be satisfactory in use. A typical
20 molecular weight is in the range 10,000 to 200,000, but others may be used if desired.

The poly-vinylpyridine may be made from a vinylpyridine using polymerisation techniques known in the art, for by example chemical or electrochemical
25 polymerisation of the vinylpyridine monomer. However, as the product is available commercially, it will usually be most convenient to obtain it from commercial suppliers.

The vinylpyridine from which the poly-vinylpyridine is derived may be any compound containing a pyridine nucleus
30 and a vinyl substituent, i.e. any pyridine containing a vinyl substituent. Most conveniently it is a mono-vinylpyridine, and usually 4-vinylpyridine is the one most accessible.

The compositions of PVC and poly-vinylpyridine can be

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made by conventional methods known in the art, using any technique known to be suitable for securing an intimate mixing of the two components. A preferred method comprises thorough mixing - especially in the presence of a mutual solvent. For these purposes, the appropriate solvent, or solvent mixture, may be chosen with regard for the known solvents for the components and their compatibility. Mixing can be carried out by other techniques, for example milling - either dry or with solvent/softeners, heat, or the like - provided that it can achieve sufficiently intimate blending of the two component polymers.

The proportions of the PVC and poly-vinylpyridine may vary over a range and thereby enable the properties of the membrane composition to be adjusted to suit individual requirements. A convenient proportion is 1 part of PVC for each part of poly-vinylpyridine, by weight, but this is not strictly necessary and suitable proportions of the poly-vinylpyridine may be in the range 20 to 80% (calculated on the total composition).

The composition may be made into membranes by any of the known and conventional methods. Most conveniently, this can be done by solution casting techniques, using solvents which dissolve the component polymers (separately or as the mixed composition) and then spreading the solution on a plate or flat surface and allowing the solvent to evaporate.

A convenient solvent is tetrahydrofuran (THF), but other solvents or mixtures of solvents which are known to be able to dissolve the component polymers (especially the PVC) may be used if desired. Solvent casting can be a convenient way for combining the mixing and the membrane-forming steps.

The thickness of the membranes can be of the order already used conventionally in the art, but may be varied

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as found most appropriate having regard for the particular mixed polymer composition being used and the conditions under which it is to be used. Thus a convenient thickness is in the range 1 to 50 μm , though larger or smaller thicknesses can be used if desired.

The mode of electrolytic analysis used to carry out the method of our invention is commonly amperometric analysis, which is well known and used in the art, for example when an enzyme electrode (i.e. containing glucose oxidase) is used to act upon glucose and produce hydrogen peroxide which is then detected at the electrode as an indirect measure of the glucose content of a sample. If desired, other known modes of analytical measurement may be used for determination of appropriate analytes - for example pulsed amperometric determination (commonly known as "PAD") may be used for the direct determination of glucose without the mediation of an enzyme.

The principal advantage of our sensors is that they can be used in conditions of variable pH and temperature without becoming irreversibly fouled; fouling is not prevented, but when it occurs it is reversible - apparently even fully reversible. This behaviour is in remarkable contrast with that of conventional membrane materials which are commonly used, which lose their usefulness because the fouling on them is not reversible. This enables the membrane materials of the present invention to be used until a level of fouling becomes sufficiently evident to interfere with use, and can then be cleaned and used again. This cycle of "use, cleaning and re-use" can be repeated several times before the efficiency of the membrane is diminished appreciably. Though cleaning to reverse fouling cannot be repeated indefinitely, we find that satisfactory results can usually be obtained for up to about 10 cleaning cycles and possibly more.

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The sensor device of our invention can have a single membrane or, if desired, be made up of multiple layers of membrane material. When multiple layers of membrane are used, these may be the same or different. When multiple
5 membrane layers are used, any membrane layer or layers other any of the wide variety of materials known in the art. Examples of these include dialysis membranes, and in general are preferably not diffusion-limiting membranes, at least to the extent that they do not limit diffusion and
10 passage of desired species towards the detecting means.

Sensor devices of the electrolytic (amperometric) type are often used for examination of biological fluids for example blood, urine, and the like. It happens that these biological fluids contain a number of interferents which --
15 even though their identity has not yet been established in all instances have the ability to interfere with the detection and determination of selected analyte components, especially (but not limited to) glucose. Some of these can be dealt with by using an enzyme system to act on selected
20 components and make the detection process more readily controlled. In particular, glucose may be determined by action of an enzyme (glucose oxidase) which generates hydrogen peroxide and that compound is then detected and used as a measure of the glucose content. High molecular
25 weight compounds (e.g. proteins) and colloidal materials can easily interfere with the action of such systems, either by blocking access of the sample components to the enzyme-containing part of a sensor or by interfering with the activity of the enzyme itself.

30 Therefore, to use the anti-fouling properties of the PVC/poly-vinylpyridine membrane formulation most effectively, the mixed membrane is used as the outermost layer so that it contacts the sample under examination. It then excludes high molecular weight materials from access

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to a detector (e.g. an electrode) -- so that is not contaminated -- and can maintain this action for a satisfactory time, up to 12 hours, and even longer. Then, when the composite membrane has become fouled and the efficiency of the sensor device is becoming impaired, the sensor device can be revived by simply removing it from any sample or sampling chamber in which it has been used and washing or rinsing it with clean water or a convenient solution, for example a saline or other electrolyte solution.

This washing procedure is very simple and convenient, and the principal precaution that a user should take is simply to ensure that the procedure does not cause any mechanical damage to the membrane. The effectiveness of the cleaning and the time when cleaning is required can be checked readily by determining the response of the sensor device to glucose (or, if appropriate, another analyte substrate) and comparing this response with that at the time of calibration or before initial use.

Thus, the sensors of our invention can exclude serum and urine interferents from a detector (especially a working electrode) in a highly effective manner, which is a valuable feature of this invention.

The mechanism by which the membrane derives its properties is not at all evident at present, but the effect is clear and very surprising. Membranes made from PVC alone are subject to bio-fouling when in use this fouling effect is not reversible by cleaning.

Poly-(4-vinylpyridine) is soluble in water, and so is not itself a candidate for use as a membrane in a sensor for use in aqueous systems, and PVC is well known to be insoluble in water. It is therefore surprising that the two components can be mixed together to form a composition which is sufficiently stable to serve as a membrane in an

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aqueous system for a reasonable time.

The polymer compositions, sensor devices and methods for their use according to this invention are especially useful for the detection and determination of sugars, especially glucose, in biological media but are not to be taken as being useful solely for this purpose. The desirable combination of bio-compatibility and permeability towards the desired analyte such as glucose make them exceptionally well suited for this.

The invention is illustrated but not limited by the accompanying Example in which the parts and percentages are by weight unless stated otherwise.

EXAMPLE.

A composite film comprising an intimate mixture of PVC and poly-(4-vinylpyridine) was prepared by first preparing a solution containing PVC (2% weight/volume) dissolved in tetrahydrofuran and adding to this solid poly-(4-vinylpyridine) in an amount which gives 2% (weight/volume) in the solution. This mixture was kept for 12 hours at ambient temperature, with agitation from time to time, to allow the solid poly-(4-vinylpyridine) to become completely dissolved.

The resulting solution of the two polymers was then coated on to the surface of a platinum electrode by dip-coating. The coated electrode was then allowed to dry in air at ambient temperature for at least 12 hours. As an alternative, the coating was carried out by dropping the solution on to the surface of the electrode.

The thickness of the deposited layer of polymer was about 50 μ m.

The coated electrode was then immersed as anode in an aqueous solution of glucose in a conventional amperometric cell, and polarising potential applied. The calibration of

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this arrangement showed a satisfactory response up to at least 35 mM glucose concentration, with a typical response being 3.5 μ A per 30 mM glucose.

For comparison, a similar electrode coated with only un-modified PVC did not permit diffusion of the glucose -- as confirmed by the low current output from it in the same cell arrangement. This showed that the composite coating of PVC and poly-(4-vinylpyridine) allowed rapid permeation of the glucose, thus giving high current responses due to the oxidation of the carbohydrate.

Next, the electrode coated with the composite coating of PVC and poly-(4-vinylpyridine) was contacted under similar conditions with aqueous solutions of glucose in which yeast cells were suspended at a concentration of 10 g (dry weight) of cells per litre. The effect of the yeast cells was to reduce the responsiveness of the electrode in measuring the glucose analyte, eventually down to 1-2 nA. This loss of responsiveness indicated that the yeast cells had de-activated the mixed-polymer membrane through binding to it and rendered it no longer useful for measurement of glucose content.

However, when the de-activated sensor was removed from the suspension of yeast cells and transferred to an aqueous solution of glucose without any suspended yeast cells, it was found that the responsiveness to the glucose was regained, i.e. the mixed polymer membrane had recovered its permeability to glucose.

This indicates that the mixed polymer membrane - though not totally immune to bio-fouling under extreme conditions - nevertheless had achieved reversibility of the bio-fouling. This is in contrast to the electrode and known membrane materials, which, under similar conditions are irreversibly deactivated.

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